NEW YORK PATHOLOGICAL SOCIETY

ABSTRACTS OF PAPERS AND DISCUSSION

Presented at the Meeting of March 28, 1963 at The New York Academy of Medicine

*Sex Chromosome Abnormalities**

ORLANDO J. MILLER

Assistant Professor of Obstetrics and Gynecology
Columbia University College of Physicians and Surgeons
Career Investigator, Health Research Council of the City of New York

Analysis of the chromosome complement, or karyotype, of an individual requires only a source of dividing cells that can be arrested in metaphase and swelled with hypotonic solution before fixation. Bone marrow, testis and some tumors contain enough mitotic figures, but such material is frequently unobtainable from the patient to be studied. Present-day tissue culture methods[1] make it possible to obtain adequate numbers of dividing cells from almost any tissue, and even from a blood sample.[2] Examination of such a single tissue will sometimes reveal chromosomal mosaicism, i.e. the presence of two or more types of cells that differ in their chromosome complement. However, examination of other tissues is sometimes necessary to show mosaicism.

Another limitation of the present methods is that only a small number of the human chromosomes are so morphologically distinctive that they can be accurately recognized.[3] The X chromosome, for example, is not distinguishable from the other chromosomes in group 6-12 + X. However, the number and size of the X chromosomes can be inferred from the sex chromatin pattern. The maximum number of sex chromatin masses in diploid cells is one less than the number of X chromosomes. When one X chromosome is larger than the normal X, the sex chromatin mass is also larger than normal. When the X chromosome is smaller than normal, so is the sex chromatin mass.[4]

Recently, a second technique has been developed that permits recognition of all the X chromosomes in a cell except one. The technique involves an autoradiographic study of chromosome duplication, using radioactively tagged thymidine, a precursor of deoxyribonucleic acid (DNA). In normal females, one chromosome in the 6-12 + X group incorporates the labeled thymidine after the other chromosomes have terminated DNA synthesis or replication. Cells from XY and XO individuals have no such late-replicating chromosome, which thus appears to be an X chromosome.[5] The number of late-replicating X chromosomes is the same as the maximum number of sex chromatin masses in normal females,[5, 6] in XXX and XXXXX females[7, 8, 9] and in XXXY and XXXXY males.[7, 9, 10] Furthermore, in individuals with larger than normal sex chromatin masses and an X-isochromosome-X cell line, the isochromosome-X is consistently the late-replicating X chromosome.[8, 9, 11, 12] This provides further evidence that the late-replicating X chromosome forms the sex chromatin mass, and thus accounts for the size relationship as well as the numerical relationship between X chromosomes and sex chromatin masses.

What kinds of patients are most likely to have abnormalities of the sex chromosomes? Obviously, those with an abnormal sex chromatin pattern. But patients with a normal sex chromatin pattern can also have a

* Supported in part by grants from the National Foundation, the U.S. Public Health Service (GM-07608 and a General Research Support Grant to Columbia University), and from the Association for Aid to Crippled Children.

sex chromosome abnormality. Females with Turner's syndrome or just primary amenorrhea, males with Klinefelter's syndrome or just testicular atrophy, and intersexes, primarily those with abnormal gonads, are likely to have a sex chromosome abnormality.

The three main types of chromosomal aberrations seen in human patients are aneuploidy, mosaicism and structural rearrangements.[12] Aneuploidy is the presence of an abnormal number of chromosomes. Nine types involving the sex chromosomes are now known. Since intersexuality is not usually present in individuals with aneuploid chromosome complements, these can be classified according to the sex of each individual. Females with XO, XXX, XXXX and XXXXX sex chromosome complements have been found. Only the XO complement is associated with abnormal sexual development. These XO individuals almost always have gonadal dysgenesis (Turner's syndrome). Males with XYY, XXY, XXXY and XXXXY sex chromosome complements have been found. Secondary testicular atrophy (seminiferous tubule dysgenesis) usually occurs in the males with two or more X chromosomes. They may have Klinefelter's syndrome, but XXXXY males and sometimes XXYY males, present rather distinctive syndromes that are clinically recognizable.

From the above summary, it is clear that the Y chromosome is all-important in sex determination. However, the Y chromosome has no influence on the sex chromatin pattern, which gives information only about the number and size of the X chromosomes. Individuals with complex chromatin patterns may have chromosome mosaicism.

Sex chromosome mosaicism is receiving increasing attention, in part because of its frequency, but also because of the extremely variable clinical picture that can occur in a series of individuals with the same combination of cell lines. New insight into the genetic control of development may be gained from studying these chromosomal mosaics. The known types of sex chromosome mosaicism include XX/XO, XXX/XO, XXX/XX/XO, XX/XY, XY/XO, XY/XO/XX, XYY/XO, XXY/XY, XXY/XX/XO, XXXY/XY and XXXXY/XXXY. Intersexuality is commonly found in individuals who have a Y chromosome in one cell line and no Y chromosome in the other. Turner's syndrome occurs not only in individuals with XO aneuploidy but also in many of the chromosomal mosaics who have an XO cell line. Klinefelter's syndrome occurs not only in XXY males but also in many of the chromosomal mosaics who have an XXY cell line. However, this syndrome does not always occur in such mosaics. Consequently, not every chromatin positive male develops testicular atrophy. This is probably due to the presence of a normal XY cell line, which permits normal testicular maturation.

Structural changes involving the sex chromosomes have been less frequently observed than aneuploidy and mosaicism. Known examples include deletion of a part of an X chromosome, a ring X chromosome, and an isochromosome X, which involves a duplication of part of the chromosome and a deletion of another portion. Such structural changes are of great interest because they have been useful in clarifying the dynamic relationship between the X chromosomes and the sex chromatin pattern.

REFERENCES

1. Ford, C. E. Methodology of chromosomal analysis in man, *National Cancer Institute Monograph No. 7*:105-17, 1962.
2. Moorhead, P. S. and others. Chromosome preparations of leucocytes cultured from human peripheral blood, *Exp. Cell Res. 20*:613-16, 1960.
3. Böök, J. A. and others. Proposed standard system of nomenclature of human mitotic chromosomes, *Lancet 1*:1063-65 (May 14), 1963.
4. Jacobs, P. A. and others. Cytogenetic studies in primary amenorrhoea, *Lancet 1*:1183-88 (June 3), 1961.
5. Morishima, A., Grumbach, M. M. and Taylor, J. H. Asynchronous duplication of human chromosomes and the origin of sex chromatin, *Proc. Nat. Acad. Sci. USA 48*:756-63 (May 15), 1962.
6. Bader, S., Miller, O. J. and Mukherjee, B. B. Observations on chromosome duplication in cultured human leucocytes. *Exp. Cell. Res. 31*: 100-12, 1963.
7. Mukherjee, B. B. and others. Autoradiographic study of chromosome duplication in cultured leucocytes from presumptive XXX and XXXXY human subjects. *Exp. Cell Res.* In press.
8. Giannelli, F. Pattern of X-chromosome deoxyribonucleic acid synthesis in two women with abnormal sex-chromosome complements, *Lancet 1*:863-65 (April 20), 1963.
9. Grumbach, M. M., Morishima, A. and Taylor, J. H. Human sex chromosome abnormalities in relation to DNA replication and heterochromatization, *Proc. Nat. Acad. Sci. USA. 49*:581-89, 1963.
10. Rowley, J. and others. Synthesis of deoxyribonucleic acid in X-chromosomes of an XXXXY male, *Nature 197*:251-52 (Jan. 19), 1963.
11. Muldal, S. and others. Tritiated thymidine incorporation in an isochromosome for the long arm of the X chromosome in man, *Lancet 1*: 861-63 (April 20), 1963.
12. Miller, O. J., Mukherjee, B. B. and Bader, S. Autoradiographic studies of the leucocytes cultured from an XO/X-isochromosome X mosaic human female. *Nature 200*:918-19, 1963.
13. Hirschhorn, K. and Cooper, H. L. Chromosomal aberrations in human disease: A review of the status of cytogenetics in medicine, *Amer. J. Med. 31*:442-70, 1961.

Chromosome Changes in Neoplasia

PETER C. NOWELL

Department of Pathology
The University of Pennsylvania School of Medicine

Recent improvements in cytogenetic techniques have been used to study the chromosomes of a variety of primary tumors. Sufficient data have now been collected to permit the following generalizations: 1) one form of human leukemia, chronic granulocytic leukemia, is characterized by a consistent specific chromosome change, an abnormal chromosome 21 (Ph^1); 2) other neoplasms studied, including the other common forms of human leukemia, the myeloproliferative syndrome in man, certain rodent leukemias, and epidermal rabbit tumors, have shown either inconstant chromosome abnormalities which varied from case to case, or no changes at all.

These findings suggest: 1) the chromosome abnormality (Ph^1) in chronic granulocytic leukemia is involved in the initiation of this neoplasm; 2) chromosome changes thus far observed in other tumors are secondary phenomena, occurring after the initiation of the neoplastic process, but perhaps involved in its progression.

DISCUSSION

CHARLES P. MILES: In attempting to bridge the gap between the chromosomes in sex and in cancer, one runs a grave risk of doing justice to neither.

I would like to say a few words about the status of chromosomal sex determination in the general area of intersex and of Klinefelter and Turner's syndromes. The most basic developmental element here seems to be in the formation of the gonads. When we have, for example, cases such as testicular feminization in which an apparently normal female habitus is accompanied by undescended testes, the sex chromosomes are XY. Clearly it would seem that the XY karyotype is determining the formation of testes, while the secondary sexual characteristics are less directly determined. Similarly, in Klinefelter's syndrome, an X and a Y chromosome determine formation of testes despite the presence of one, two, or more surplus X chromosomes. Conversely in Turner's syndrome, the presence of only one X chromosome determines rudimentary ovarian structures if any gonads are formed. It is also interesting that, so far as I know, no cases of complete sex reversal have been reported. By this I mean individuals with XY chromosomes who have a completely normal female anatomy with normal ovaries, or conversely, XX chromosomes in an otherwise normal male.

It is interesting, too, as Dr. Miller has pointed out, that contrary to what we might have expected in advance, the chromosomes have not helped us very much in so-called true hermaphroditism. The cases of true hermaphroditism that have been analyzed have shown usually one situation or another, either XX or XY, although many of these cases were analyzed at a relatively early date, before the problem of mosaicism was completely understood, and possibly some of them are mosaics, but in general we can't account for these cases on the basis of chromosomal abnormality per se.

In this connection there is another problem which I think has been insufficiently emphasized. The gonads have been described in a rather cursory way such as streak gonads, gonads showing ovarian-like stroma with Leydig cells, etc., without a sufficiently precise description, and it is very hard therefore to evaluate these cases. A stricter anatomical classification of these rudimentary gonads might shed more light on the relationship between the gonadal development and the karyotype.

Now let me turn to the problem of neoplasia. Dr. Nowell has mentioned Boveri's

theory. Boveri observed that there were abnormal chromosomes in cancer and abnormal metaphase patterns, and he inferred from this that perhaps these abnormalities led to the development of cancer. I think it is well to bear in mind just exactly what Boveri believed. Apparently he believed that there were so-called chromosomal mutations in tumors. Now mutations may be divided into two kinds, chromosome mutations and point mutations. In a chromosome mutation there is a visible abnormality in the chromosome itself, usually involving a deletion or a rearrangement of segments. With a point mutation you have some change in the phenotype, but there is no cytologically visible change in the chromosomes.

There are other kinds of genetic changes that we might think about that are not necessarily either point or chromosome mutations. Now what is the chromosome status in neoplasia? We have already heard about chronic myeloid leukemia. Just when many of us had believed that the Boveri theory, the chromosome mutation theory of carcinogenesis was moribund, Dr. Nowell and his colleagues have revived it. But it does seem to be a very unusual case. The other leukemias, as he has pointed out, and other investigators have found, may show predominantly normal karyotypes but with some greater than normal variation. Now there seem to be a few other kinds of chromosomal situations in neoplasia and specifically in the solid tumors. In the first place there is the situation to which Dr. Nowell has alluded, of having very gross abnormalities in the karyotypes. There are karyotypes that have 70-80 chromosomes, and individual chromosomes may be markedly abnormal. Now this is what is generally conceded to be the situation in solid tumors. I think there are other situations but they are rather more difficult to analyze.

Now let me diverge by mentioning some of the technics that can be used for analysis of the chromosomes, especially in solid tumors. Most of them have been analyzed with squash preparations. Usually the squash preparation, which simply involves exactly what it says, squashing the dividing cells by flattening them under a cover slip, has been done on body effusions, and it may be noted that this restricts rather severely the number of tumor types that can be examined. It is a strong selecting factor because we can only examine those tumors which are characteristically found in pleural or peritoneal effusions.

The second method involves squashes of solid tumor tissue and these, as most investigators concede, tend to give very poor results. It is very difficult to get good metaphase spreads.

Another technic is tissue culture. My first slide shows a relatively small lung tumor, and to that extent perhaps an early tumor. It is growing in tissue culture more or less in a monolayer, and you can see that there are very marked variations in the size of the nuclei and in the number of chromosomes from one metaphase to the next. The next slide shows another figure, obviously uncountable, but at any rate with a great many chromosomes. So this is one type of situation in tumors and perhaps the most common variety of those that have been analyzed.

The next slide shows a thyroid carcinoma which is metastatic in a lymph node. The lymph node is solidly filled with tumor and in the next slide we see the result of growing a portion of this carcinoma in tissue culture. It shows a monolayer of cells which, in contrast to what we saw before, show rather uniform nuclei. There is a solitary mitotic figure and in general there is a low mitotic rate such as is not uncommon in thyroid carcinomas. The next slide shows a karyotype based on a very small number of counts in this thyroid carcinoma, and this appears to be a normal karyotype. Unfortunately only eight cells were countable in this situation. Roughly three of them were 45, and five were 46. In short, one may have normal karyotypes in solid neoplasms.

Now another technic we have used lately on tumors is a variant of the air dry method which is usually used with peripheral blood cultures. In this case we are using it on a series of solid tumors that have been carried in animals for periods up to ten years. These are tumors maintained at the Sloan-Kettering Institute by Dr. Helene Toolan. Some of these tumors show the characteristic patterns usually described for

solid tumors. They show a variation in the number of chromosomes from cell to cell. The next slide shows such a figure with about 56 chromosomes. Here I would like to point out one of the difficulties. This does not give as nice a preparation. The chromosomes are not as well delineated as they are in normal fibroblasts or normal blood cells in culture. This, I am inclined to believe, is a characteristic feature of tumors. The advantage of this particular preparation is that you have a very tight cluster so that you are fairly sure no chromosomes have been lost.

Now in the next slide there is a figure from the same tumor. It has about 57 chromosomes but they are so well spread that one may suspect that there has been some breakage. This seems to be a general problem which must be borne in mind in considering reports analyzing either effusions or solid tumors; namely, that the best preparations or most easily analyzed preparations are most apt to be broken with a resultant loss of chromosomes.

The next slide shows an epidermoid carcinoma of the cervix which had been growing for many years. Once again the chromosomes are easier to count this way but they may be broken. In the next slide we have a tumor that does not spread as nicely as this epidermoid carcinoma of the cervix. This happens to be a rather unusual tumor to begin with. It is a sarcoma and interestingly enough so far it looks as if all of the cells in this tumor have 47 chromosomes. This is not, however, like Klinefelter's syndrome, since this karyotype is markedly abnormal. We have done some karyotypes and there appears to be the same chromosome complement in every cell.

The next slide illustrates a tumor which has been carried in rats and hamsters for nine years. It was an embryonal rhabdomyosarcoma, an uncommon tumor which in this case was even less common in arising in a 37-year-old man. We have rather poor results on this tumor as yet, but nevertheless the counts suggest that it has 46 chromosomes, and appears to show a normal karyotype. So here is a second tumor that apparently has a normal karyotype.

Now, perhaps I might conclude by mentioning the other side of the story, the viral theory as opposed to the chromosome or somatic mutation theory of tumor origin. Recently, several investigators have reported the effect of SV 40 virus on cell cultures. This virus appears to be able to induce chromosome abnormalities which are very similar to some of the abnormalities one sees in the majority of human tumors. There is another report involving Herpes virus which, in a line of hamster cells, also induces chromosome abnormalities. It remains to be seen how closely these reports apply to the question of cancer, but they may provide the first cytologic link between viruses and human cancer.